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By: Leslie Lindsay

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Rex Bitner et al.	Docket No.	016026/9038
Serial No.	09/475,958	Group Art Unit	1655
Filed:	December 30, 1999	Examiner:	B. Sisson
For:	CELL CONCENTRATION AND LYSATE CLEARANCE USING PARAMAGNETIC PARTICLES		

AMENDMENT AND REQUEST FOR RECONSIDERATION 37 CFR 1.111

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

In the matter of the above-identified application, and in response to the Office Action mailed August 8, 2001, Applicants submit herewith on separate pages a clean copy of Amendments, a marked version of the claims clearly showing the changes, and Remarks. Applicants respectfully request entry of the amendments, reconsideration on the merits of the application, and allowance of the claims.

AMENDMENTS

In the claims:

8. A method of clearing a solution of disrupted biological material other than target nucleic acids, according to steps comprising:

(a) providing a solution comprising a disrupted biological material;

(b) combining the solution with magnetic particles under conditions wherein the disrupted biological material other than target nucleic acids selectively adsorbs directly to the particles, thereby forming a complex, wherein said magnetic particles are selected from the group consisting of (1) pH dependent ion exchange particles and (2) silica magnetic particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface; and

(c) separating the complex from the solution by application of magnetic force.

REMARKS

Claims 1-25 and 27-29 are pending in the application. In a non-final Office Action mailed August 8, 2001, claims 1-6, 8-18, 20-25, and 27-29 were rejected under 35 U.S.C. 102(e) as being anticipated by Smith *et al.*; claims 8, 9, 18, and 19 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* in view of Sosnowski *et al.*

In view of the amendments above and the arguments below, Applicants respectfully request reconsideration on the merits of the application, and allowance of the claims.

Rejections under 35 U.S.C. 102(e)

Claims 1-6, 8-18, 20-25, and 27-29 stand rejected under 35 U.S.C. 102(e) as being anticipated by Smith *et al.*(U.S. Patent No. 6,027,945). The Examiner characterized Smith *et al.* as disclosing the use of magnetic silica particles for the direct immobilization of nucleic acids from various sources, including mammalian tissue and blood, and bacteria (column 9 through column 10). Smith *et al.* was further characterized by the Examiner as teaching a two step method of separating non-nucleic acid materials and nucleic acids from the sample (column 11). The Examiner asserted that the teachings of Smith *et al.* meet the limitations of claims 1-6, 8-18, 20-25, and 27-29.

Applicants respectfully submit that, for the reasons set forth below, Smith *et al.* does not teach all of the claim limitations of claims 1-6, 8-18, or 27-29, and therefore, does not anticipate the claims.

Claims 1-6

Claim 1, from which claims 2-7 depend, is set forth below:

> 1. A method of using magnetic particles to concentrate or harvest cells, comprising the steps of:
>
> (a) combining cells with magnetic particles, under conditions wherein the cells selectively adsorb directly to the particles thereby forming a complex, wherein said magnetic particles are selected from the group consisting of (1) pH dependent ion exchange particles and (2) silica magnetic particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface; and
>
> (b) isolating the complex from the solution by application of magnetic force.

Claims 1-6 are drawn to a method of concentrating or harvesting cells using magnetic particles under conditions wherein the cells selectively adsorb to the particles, the particles selected from pH-dependent ion-exchange particles and silica magnetic core particles

consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface. In contrast, Smith *et al.* is drawn to isolating biological target materials, particularly nucleic acids such as DNA or RNA (see abstract; column 7, line 66-column 8, line 11). Smith *et al.* does not teach or suggest the possibility of using magnetic particles to isolate cells. Smith *et al.* notes that in order to isolate biological target material, such as nucleic acids, the material must be present in a form that is available to adhere to silica magnetic particles in the first step of the method. In fact, Smith *et al.* teaches that when the material is in a cell, the material is not available and the cells must be lysed before the isolation methods of the invention are applied (column 10, lines 3-6; column 10, lines 24-33). Smith *et al.* does not teach or suggest using any magnetic particles to concentrate or harvest cells, let alone the pH-dependent ion exchange particles or silica magnetic particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface required by claims 1-7.

Applicants respectfully submit that Smith *et al.* does not teach or suggest all of the limitations of claim 1, and therefore, neither claim 1, nor any of its dependent claims 2-7 is anticipated by Smith *et al.* Applicants request that the rejection of claims 1-6 under 35 U.S.C. 102(e) be withdrawn.

Claims 8-18

Amended claim 8, from which claims 9-20 depend, reads as follows:

> 8. A method of clearing a solution of disrupted biological material other than target nucleic acids, according to steps comprising:
>
> (a) providing a solution comprising a disrupted biological material;
>
> (b) combining the solution with magnetic particles under conditions wherein the disrupted biological material other than target nucleic acids selectively adsorbs directly to the particles, thereby forming a complex, wherein said magnetic particles are selected from the group consisting of (1) pH dependent ion exchange particles and (2) silica magnetic particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface; and
>
> (c) separating the complex from the solution by application of magnetic force.

Claim 8 has been amended to clarify that the method clears the solution of disrupted biological materials other than the target nucleic acid in the solution. Support for this amendment is found throughout the specification (e.g., page 10, lines 12-21). Reference to

"second" magnetic particles has been deleted for purposes of clarity. The original claim recited no "first" magnetic particles. Recitation of "second magnetic particles", without reference to first magnetic particles, makes the claims somewhat confusing. In the specification, when magnetic particles are used to both clear a solution of disrupted biological material and to isolate a target nucleic acid, particles used to clear a solution of disrupted biological material are referred to as "first magnetic particles", and particles used to isolate the target nucleic acid are referred to as "second magnetic particles" (please see page 14, lines 9-14; page 5, lines 27-30; and page 6, lines 8-11). Therefore, Applicants have deleted the adjective "second", because the use of "second magnetic particles", as defined in the specification, is inconsistent with the objective set forth in the preamble of the claim, i.e., to clear a solution of disrupted biological material other than target nucleic acid. As clarified by the amendment to claim 8, claims 8-20 are drawn to methods of clearing a solution of disrupted biological material other than target nucleic acid that includes the step of combining the solution with magnetic particles selected from pH-dependent ion-exchange particles and silica magnetic core particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface under conditions wherein disrupted material other than target nucleic acids selectively adsorbs directly to the particles to form a complex. The magnetic particles with the unwanted disrupted biological material attached thereto are removed to leave a cleared solution comprising the target nucleic acid.

As discussed in Smith *et al.*, the desirability of removing unwanted cellular debris prior to isolating nucleic acids is known to the art (column 10, lines 26-33). Smith *et al.* describes methods of removing cellular debris (column 10, line 50-column 11, line 8), none of which include using magnetic particles under conditions that cause selective adsorption of disrupted biological material other than target nucleic acid, as required by claims 8-18. Smith *et al.* does not discuss using magnetic particles to clear a solution of disrupted biological material, but rather, discusses isolating nucleic acid material from a cell lysate that has already been cleared of cellular debris other than DNA by one of the methods disclosed at column 10, line 50-column 11, line 8. Applicants respectfully submit that Smith *et al.* does not teach or suggest all of the limitations of claim 8, and therefore, neither claim 8, nor any of its dependent claims 9-18 is anticipated by Smith *et al.*

Claims 21-25 and 27-29

Claim 21, from which claims 22-25 and 27-29 depend, is reproduced below:

21. A method of isolating a target nucleic acid from a disrupted biological material, comprising the target nucleic acid, a first non-target material, and a second non-target material, comprising the steps of:

(a) combining a solution of the disrupted biological material with first magnetic particles under conditions wherein the first non-target material selectively adsorbs directly to the particles, thereby forming a first complex, wherein said magnetic particles are selected from the group consisting of (1) pH dependent ion exchange particles and (2) silica magnetic particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface;

(b) separating the first complex from the solution of disrupted biological material by application of magnetic force, forming a cleared solution comprising the target nucleic acid and the second non-target material;

(c) combining the cleared solution with second magnetic particles under conditions wherein the target nucleic acid adsorbs to the second magnetic particles, forming a second complex;

(d) isolating the second complex from the cleared solution;

(e) washing the second complex by combining the second complex with a wash solution and separating the second complex from the wash solution by magnetic force; and

(f) combining the washed second complex with an elution solution, under conditions wherein the target material is desorbed from the second magnetic particles.

The Examiner asserted that Smith *et al.* teaches "the aspect of performing a two-step method of separating non-nucleic acid materials and then nucleic acids from the sample is disclosed at column 11" of Smith et al. However, Smith *et al.* teaches first removing cell debris to form a cleared lysate, which is then contacted with the silica magnetic particles in second step to form a complex with the nucleic acid material (column 11, lines 19-42). Smith *et al* .discloses examples of how the cleared lysate may be formed (column 10, line 50-column 11, line 8), none of which includes removing cell debris by contacting the lysate or disrupted cells with a magnetic particles selected from silica magnetic particles consisting essentially of a magnetic core coated with a siliceous oxide having a hydrous siliceous oxide adsorptive surface or pH-dependent ion exchange magnetic particles, to form a complex of the particle and disrupted biological material, removing the complex to form a cleared solution, followed by combining the cleared solution with second magnetic particles to form a complex of target nucleic acid and magnetic particles as is required in claim 21.

Applicants respectfully submit that Smith *et al.*does not teach or suggest all of the limitations of claim 21, and therefore, neither claim 21, nor any of its dependent claims 21-25 or 27-29 is anticipated by Smith *et al.*

Rejections under 35 U.S.C. 103(a)

Independent claim 8, and claims 9, 18, and 19, which depend from claim 8, stand rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.*in view of Sosnowski *et al.*(U.S. Patent No. 6,051,380). Claim 8 was reproduced above. Claim 9 requires that the disrupted biological material is a bacterial cell lysate; claim 18 requires that the disrupted biological material of step a of claim 8 comprises cells that are first isolated by complexing with pH-dependent ion exchange magnetic particles and then disrupted; claim 19 requires that the pH-dependent ion exchange magnetic particles of claim 18 are selected from the group consisting of glycidyl-histidine modified silica magnetic particles, and glycidyl-alanine modified silica magnetic particles. Smith *et al.* is cited for the teachings set forth above in the section entitled "Rejections under 35 U.S.C. 102(e)". The Examiner acknowledged that Smith *et al.* does not to teach "the use of an amino acid coating". Sosnowski *et al.* is cited as teaching "the use of compounds such as histidine or histidine peptides, that help maintain the positive charge of the surface and thereby maintain the stability of the bound nucleic acids" (column 12). Applicants are somewhat unclear how the particular combination of references form the basis for rejecting the claims under 35 U.S.C. 103(a), and note that none of claims 8, 9, 18 or 19 require an amino acid coating.

A *prima facie* case of obviousness requires: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) the art reference or combination of references must teach all of the claim limitations (MPEP 2142).

Applicants respectfully submit that the Examiner has not established prima facie case of obviousness because Smith *et al.* and Sosnowski *et al.* do not combine to teach all of the claim limitations of independent claim 8, nor of claims 9, 18, or 19. Claim 8, from which claims 9, 18, and 19 depend, provides a method for forming a cleared solution which includes the step of forming a complex between magnetic particles and the disrupted biological material. As discussed in detail in the preceding section, Smith *et al.* teaches that bacterial lysates should be cleared prior to contacting the target nucleic acid with magnetic particles, but does not teach or suggest using magnetic particles to form complexes with disrupted

biological material other than target nucleic acid in preparing the cleared solution, as required by claims 8, 9, 18 and 19. Sosnowski *et al.* does not cure the deficiencies of the primary reference. Sosnowski *et al.* relates generally to methods and procedures for analyzing nucleic acids, specifically, a microelectronic based nucleic acid array that uses electric fields to control transport, hybridization, and stringency of nucleic acid interactions; it does not relate to methods of clearing a solution of disrupted molecular biological material other than the target nucleic acid. The Examiner noted column 10, which discusses an embodiment of the invention in which improved permeation layers containing histidine, histidine peptide, polyhistidine, lysine, lysine peptides, or other cationic compounds or substances are used. Equilibration of permeation layers with buffers containing histidine peptides (Example 13) may help maintain the stability of DNA hybrids formed between the single-stranded target nucleic acid and the complementary, immobilized capture oligonucleotides by specific base pairing between the capture oligonucleotide and the target nucleic acid. Neither Smith *et al.*nor Sosnowski *et al.*discloses a disrupted biological material that is formed by complexing cells with pH-dependent ion exchange magnetic particles or glycidyl-alanine or glycidyl–histidine modified silica magnetic particles, and then disrupting the cells, which is required by claims 18 and 19, respectively. The permeation layer of Sosnowski et al. comprising the aforementioned amino acids or peptides overlies an electrode, and serves to separate the attached or tethered oligonuclotides and hybridized target DNA sequences from the highly reactive eletrochemical environment generated at the electrode surface (please see column 7, lines 40-60). The amino acid buffers disclosed in Sosnowski are used in hybridization analysis to stabilize the DNA hybrid, whereas the method of claim 19 involves forming a cleared solution by combining a solution of disrupted biological material (disrupting cells, the cells first isolated by forming a complex between glycidyl-alanine or glycidyl–histidine modified silica magnetic particles) and removing disrupted biological material other than target nucleic acids by forming a complex between pH-dependent ion exchange magnetic particles or glycidyl-alanine or glycidyl–histidine modified silica magnetic particles.

Applicants respectfully submit the a prima facie case of obviousness has not been established because the claims do not combine to teach all of the claim limitations. Applicants request that the rejection be withdrawn.

The application is now in condition for allowance. Applicants respectfully request allowance of the claims.

Applicant notes that this response is being filed prior to the expiration of the shortened statutory period for filing a timely response. No fee is believed due in connection with this response. However, if a fee is owed, please charge such fee to Deposit Account No. 50-0842.

Respectfully submitted,

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